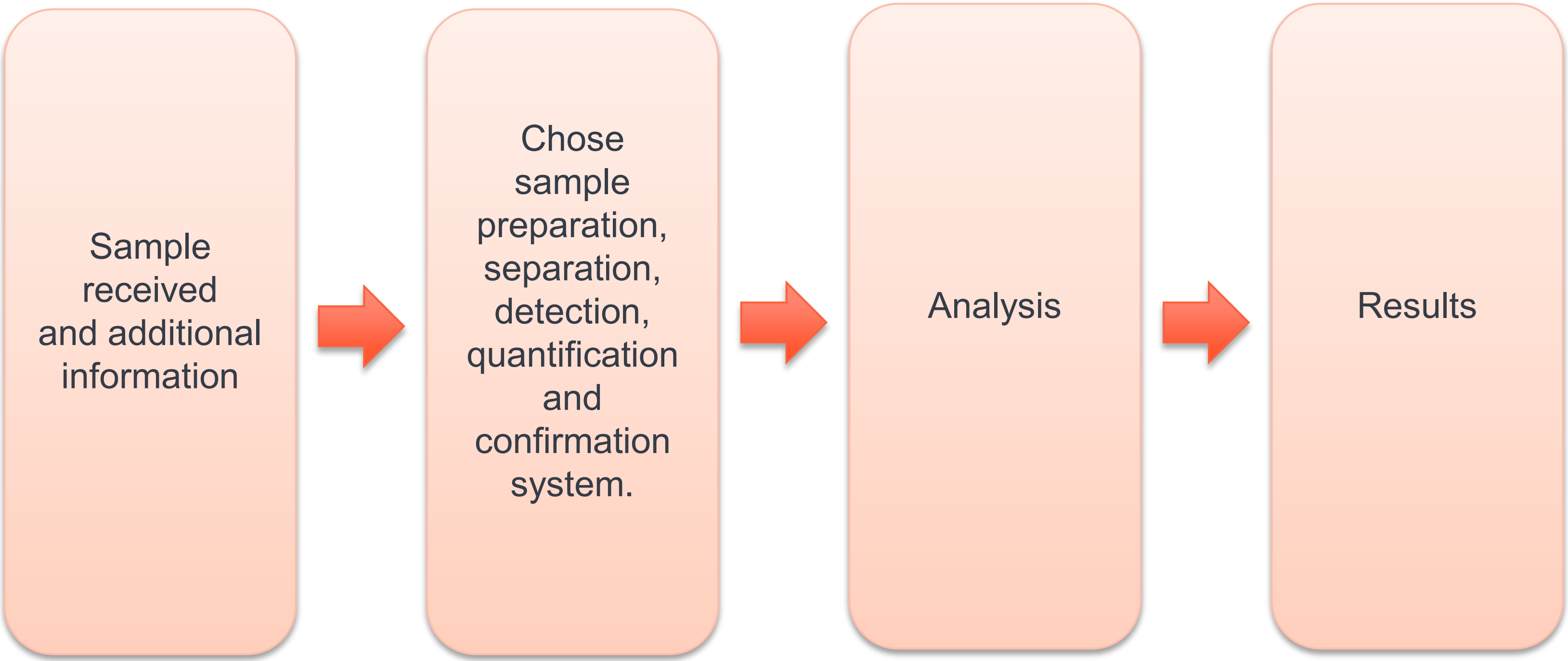
How to select the right chromatographic technique

25 09 2024

Fabio Venegoni

Method choice



Sample preparation methods

- Direct injection;
- Filtration/Centrifugation;
- Dilution;
- Solvent extraction;
- Static headspace;
- Derivatization;
- Solid Phase Micro-Extraction (SPME);
- Solid Phase Extraction (SPE):
    - Clean-up;
    - Fractionation;
    - Trace enrichment/concentration.

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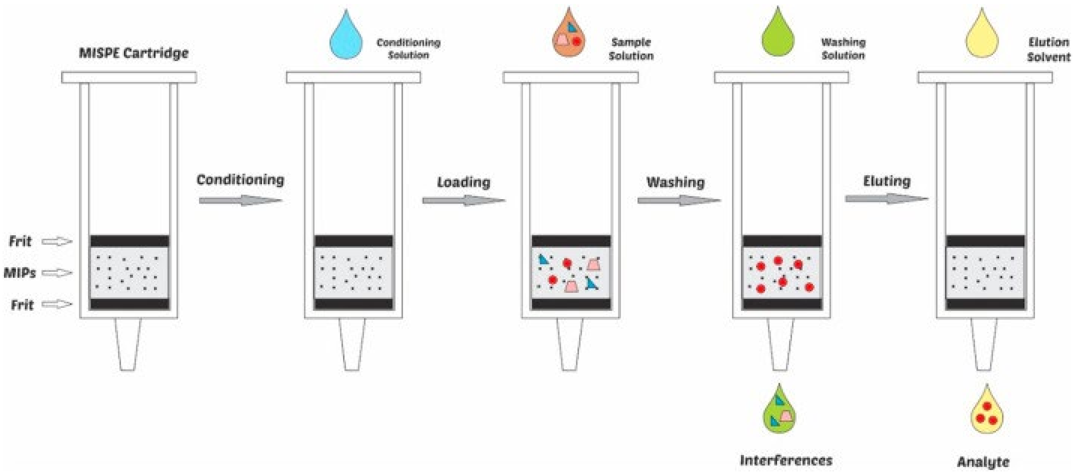
Solid Phase Extraction (GC and LC)

This sample preparation technique uses solid particles (usually contained in a cartridge) to chemically separate the different components in liquid samples, enabling the extraction, clean-up, fractionation and concentration of analytes prior to their quantification. SPE is often used for the selective removal of interferences and/or for trace concentration of very low level compounds.



Conditioning and equilibration of the sorbent in the cartridge

↓

Loading of the (pre-treated) sample

↓

Washing to remove impurities

↓

Eluation step

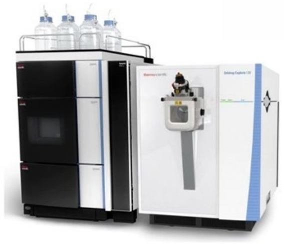
↓

GC and/or LC analysis

Separation and detection systems

Liquid Chromatography

- UV-Vis;
- MS Single Quadrupole;
- Tandem MS/MS;
- High Resolution Mass Spectrometry.



Gas Chromatography

- Flame Ionization Detector (FID);
- Thermal Conductivity Detector (TCD);
- Electron Capture Detector (ECD);
- Ionization source (EI/CI);
- MS Single Quadrupole;
- High Resolution Mass Spectrometry.

Ion Chromatography

- Conductivity;
- MS Single Quadrupole.

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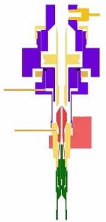
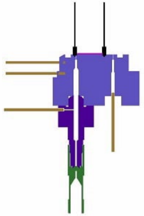
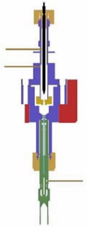
Detectors for GC and LC



Flame Ionization Detector: the most widely used detector because of its reliability, versatility, and ease of use. It responds to virtually any organic compound while generating no signal for common carrier gases.



Thermal Conductivity Detector: universal detector that responds to virtually any compound, excluding the carrier gas. It is generally used for the GC analysis of compounds that do not respond well to a FID or for concentrated samples.



Electron Capture Detector: provides trace-level analysis of halogenated organic compounds and aromatic pollutants.

Diode Array Detector (DAD): the UV absorption of the effluent is continuously measured at single or multiple wavelengths. These are by far most popular detectors for LC.

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Mass Spectrometer equipment

Mass Spectrometer is an instrument designed to separate gas phase ions according to their m/z (mass to charge ratio) value. The separation of these gas phase ions, produced by a variety of ionization methods, is achieved within the mass spectrometer using electrical and/or magnetic fields to differentiate ions.

The molecule is ionized in the ion source and the mass to charge ratio (m/z) of the ion(s) is determined by the mass analyzer. The detector generate a signal proportional to the abundance of the ion(s).

Fragmentation can occur in the ion source and/or in different stages of the mass spectrometer.

LC-MS/MS Triple Quadrupole

GC-MS Single Quadrupole

Low resolution mass analyzer: Quadrupole (Q), Ion Trap (IT) (PFOA m/z = 413)

High resolution mass analyzer: Time of Flight (ToF), Orbitrap (PFOA m/z = 412.9711)

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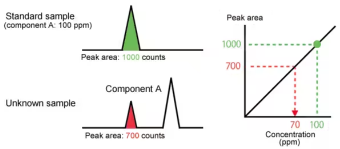
Methods for quantification and confirmation

Quantification

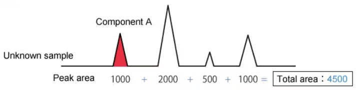
- Percentage peak area method;
- Normalized percentage peak area method;
- One point calibration;
- External standard;
- Internal standard;
- Isotope dilution.

Confirmation

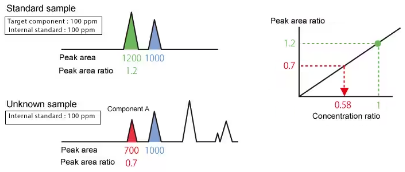
- Retention time (RT);
- Parent ion;
- Quantification/qualification transitions;
- High resolution mass determination.



Component A concentration is **70 PPM**



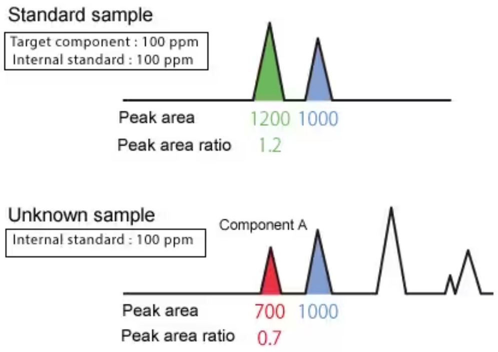
Component A concentration is **1000/4500 = 22.2 %**

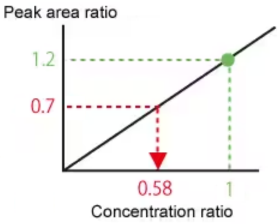


Concentration of target component in unknown sample
100 ppm (concentration of internal standard in unknown sample) × 0.7 / 1.2 = 58 ppm
Component A concentration is **58 PPM**

Methods for quantification: Internal Standard

The internal standard method calculates the target component concentration based on the relationship between the peak area ratio and concentration ratio of the target component and an internal standard.

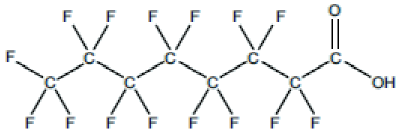




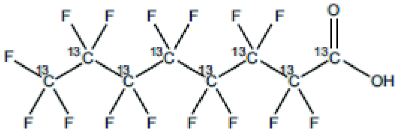
Concentration of target component in unknown sample

100 ppm (concentration of internal standard in unknown sample) × 0.7 / 1.2 = 58 ppm

Component A concentration is **58 PPM**



PFOA (414 amu)



SIL-PFOA (422 amu)

Stable Isotope Labeled Internal Standard (SIL-IS)

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Guidelines for method choice

Physic-chemistry properties of the analyte

Concentration of the analyte

Composition of the matrix

Number of samples to be analyzed

Purpose of the analysis

Chosen sample preparation / separation / detection / quantification / confirmation system

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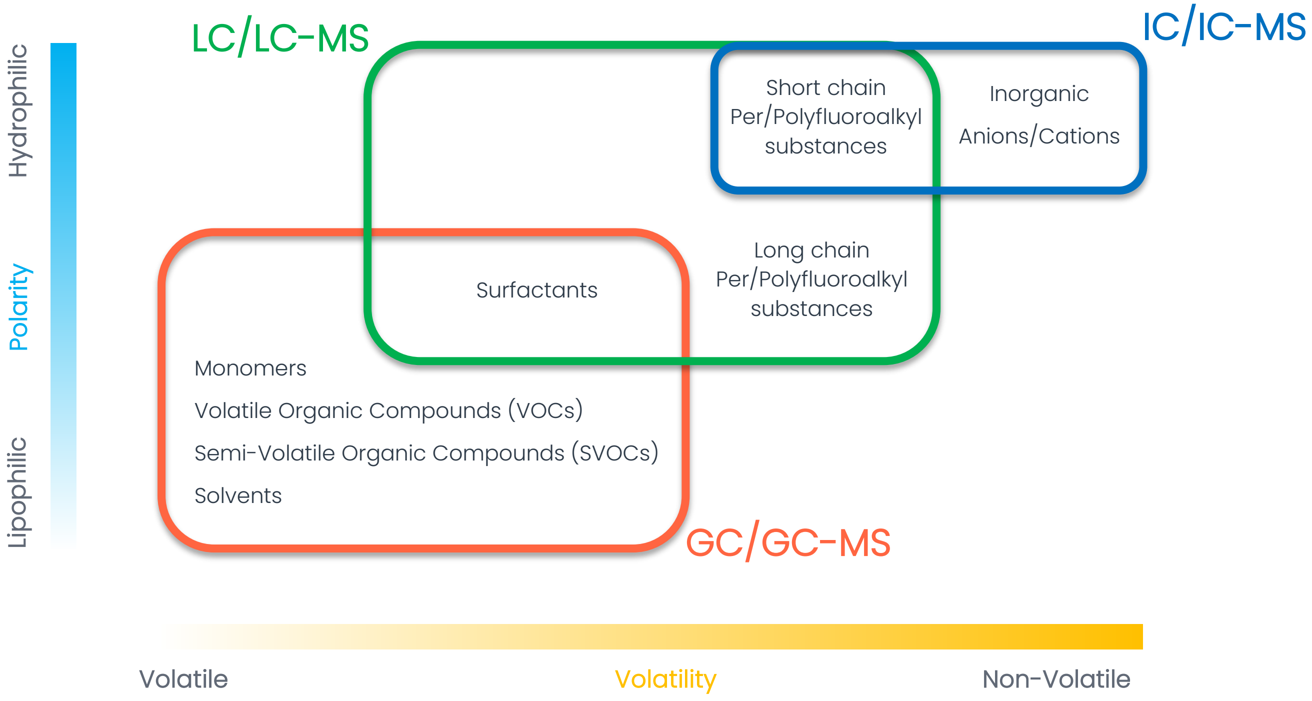
Physic-chemistry properties of the analyte



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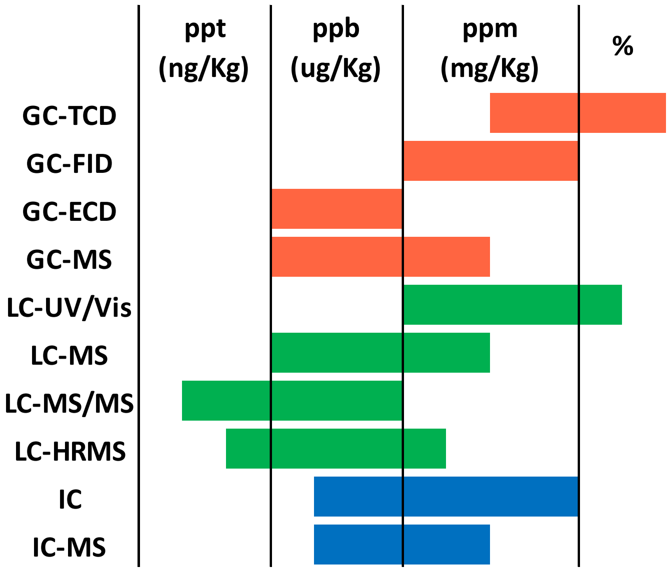
Concentration of the analyte: detection system



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Concentration of the analyte: sample preparation

Concentration higher than operative range	→	Dilution (sample pre-treatment if necessary)
Concentration in the operative range	→	Direct injection (sample pre-treatment if necessary)
Concentration lower than operative range	→	Trace enrichment/concentration (sample pre-treatment if necessary)

Composition of the matrix: sample preparation

The necessity to remove matrix components from the sample to be processed is normally the most important reason to built in sample preparation steps. In the most cases is the only possibility to ensure sufficient selectivity and sensitivity.

More simple

- Direct injection
- Dilution
- Filtration/Centrifugation
- Matrix precipitation
- Solvent extraction
- Static headspace
- Solid Phase Micro-Extraction (SPME)
- Solid Phase Extraction (SPE)

More complex

Less selective

Lower clean-up and concentration

More selective

Higher clean-up and concentration

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Composition of the matrix: quantification

The composition of matrix and the complexity of sample preparation impact the quantification approach to be used. Generally, the more complex the matrix and sample preparation, the more the capability required to monitoring recovery and matrix effects. So the use of internal standard(s) added at one or different sample preparation steps should be considered.

More simple

External standard

Lower capability to monitoring and compensate recovery and matrix effects

Internal standard

Isotope dilution (only for MS)

Higher capability to monitoring and compensate recovery and matrix effects

More complex

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Number of samples to be analyzed: sample preparation

Sample preparation in most cases is the time-limiting step in the analytical procedure. The choice of the sample preparation procedure should take into account also the number of samples to be processed and the timing of the request, with the best balance between time, cost, clean-up and technical possibility.

Purpose of the analysis: confirmation

Lower — Confidence — Higher

RT	RT + Parent ion	RT + MS/MS or HRMS
GC-TCD	LC-MS Single Quadrupole	GC-EI-MS Single Quadrupole
GC-FID	IC-MS Single Quadrupole	LC-MS Triple Quadrupole
GC-ECD		LC-HRMS
LC-UV/Vis		
IC-Conductivity		

Generally, the higher the impact of a wrong result, the higher the confidence required. High level of confidence generally require more complex, time consuming and expensive techniques.

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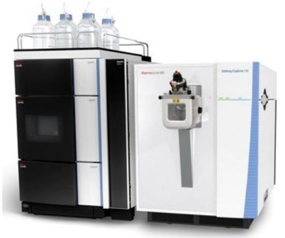
Purpose of the analysis: qualitative analysis

When the purpose of the analysis is the characterization of the constituents of a sample or the identification of unknown compounds, the highest capability to obtain information from the molecules is needed. So, chromatographic techniques coupled with mass spectrometer detector are the best choice for qualitative analysis.

GC-EI-MS Single Quadrupole

GC-HRMS



LC-HRMS

IC-MS Single Quadrupole

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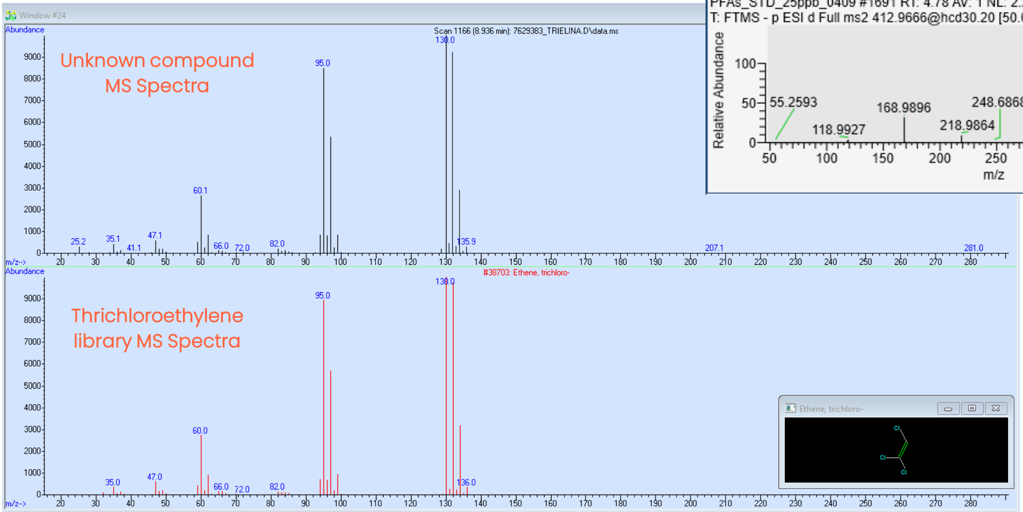
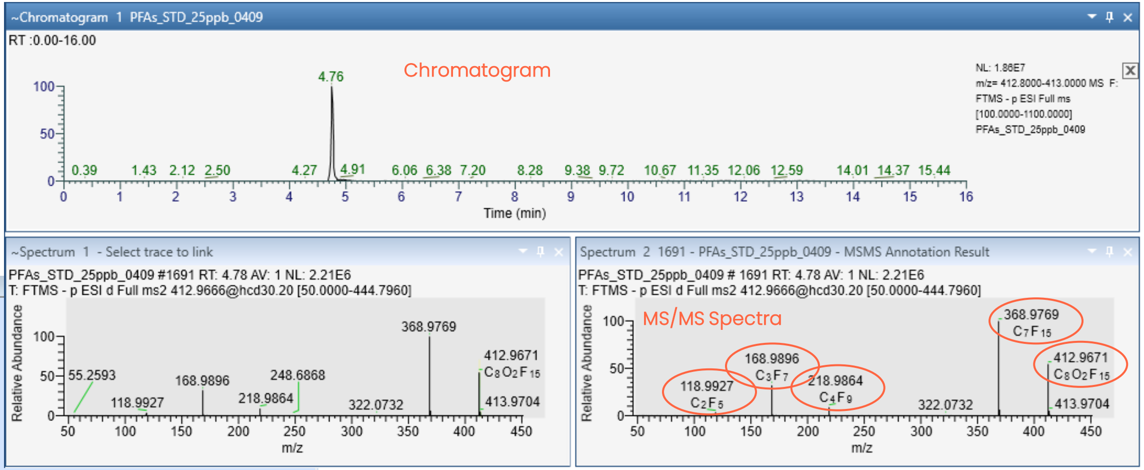
Purpose of the analysis: qualitative analysis

Trichloroethylene identification by GC-EI-MS Library tool





PFOA identification by LC-HRMS elemental composition tool.

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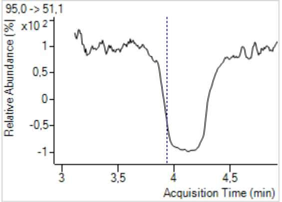
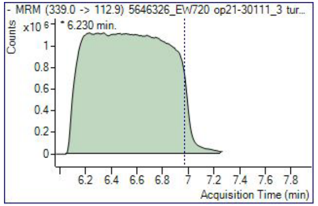
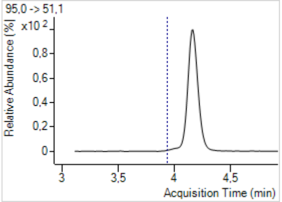
Consequences of a wrong choice…



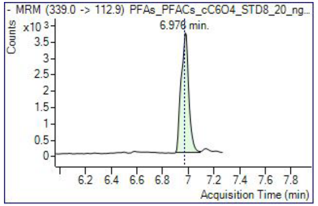
Incorrect sample clean-up with strong matrix effect (underestimation!)



Incorrect concentration estimation: fouling of the analytical system!

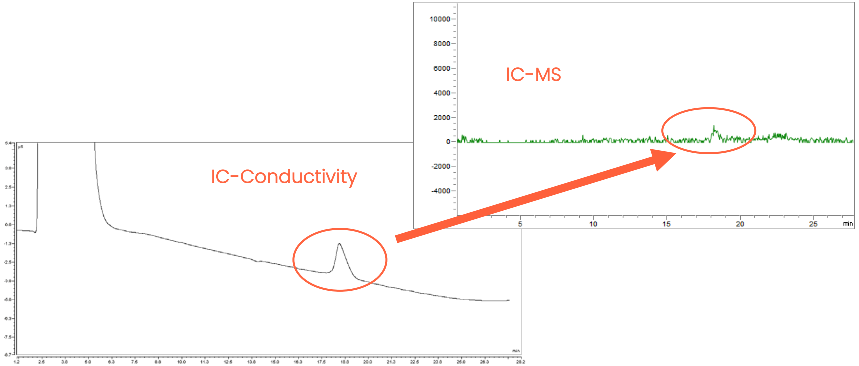


Correct sample clean-up, no matrix effect.



Concentration of the analyte in the higher standard solution.

Impurity at the same retention time of the target analyte: detection by IC-Conductivity but not by IC-MS.



Thank You